

Chapter 19

Dynamics of Quenching by Plasma from Patients with Various Pathological Conditions

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INTRODUCTION

In the last few years, techniques have been described which permit the direct incorporation of plasma into organic scintillation mixtures by using tensioactive agents.¹⁻³ Many studies have been perfected on the physical characteristics of these heterogeneous systems. A great number of these studies emphasize the difficulties encountered with sample stability,^{4,5} and with the measurement of detection efficiency^{6,7} when using the classical methods of quenching correction with these systems.⁸⁻¹⁰ Perhaps, due to these difficulties, the use of tensioactive agents in clinical laboratories working with β -emitters has not been well accepted and clinical assays on patients with these nuclides are usually accomplished with complex physical or chemical treatment of plasma samples.

Clinical tests with β -emitters are shown in Table 1 together with the corresponding preparative method most currently employed for each test.

Our aim has been to perfect a reliable method for the preparation of plasma samples while benefiting from the inherent simplicity obtained through the use of tensioactive agents. We have developed, for clinical studies, a simple method for counting untreated plasma in conjunction with tensioactive agents.

In the first part of this communication, the methods used for direct plasma sample preparation, counting efficiency measurement and quench correction are described. In the second part, we discuss our results which show that at least for clinical studies, counting efficiencies vary widely. It is shown that this quenching is due to the colour changes in plasma samples. Finally, the practical consequences of counting and the calculation of d.p.m. from c.p.m. in labelled plasma samples are discussed.

MATERIALS

All these studies have been conducted with an SL 40[†] liquid scintillation counter, equipped with an 'on-line' computer. The coefficients of the quenching curves were com-

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Table 1. Clinical studies with the aid of β -radioisotopes.

Designation	Tracer	Molecule	Plasma processing	Minimal number of plasma samples required
Whole body water	Tritium	Water	Plasma protein precipitation. Combustion	2
Extracellular (sulphate) space	Sulphur-35	SO ₄ ⁻⁻	Precipitation, isolation with ion exchange resin	4
Calcium metabolism	Calcium-45	Ca ⁺⁺	Complex	6
Iron metabolism	Iron-55	Fe ⁺⁺	Complex	8

puted with a PDP 10 computer. § The various products used for sample preparation were:

1. toluene
2. Triton X-100®*
3. PPO†
4. 2,5-POPOP†

For quenching studies we have used haemoglobin or Hank's Liquid which is red at neutral pH and yellow at acid pH. For chemical quenching studies we have made use of pyridine which is water-soluble. The plasma samples were collected from patients suffering from various diseases which may be described here as:

1. nephrotic syndrome
2. hepatic syndrome

The various studies currently performed with β -tracers in our laboratories are represented in Table 2.

METHODS

Plasma sample preparation

The method used for plasma sample preparation is the same one as we have previously described¹¹ and is schematically represented in Table 3. This is a direct incorporation in a toluene-Triton mixture but with a dilution of plasma in physiological saline. This procedure results in a better stability of the plasma components and permits detection efficiency determination with the aid of the 'pseudo-internal' method as described below.

Counting efficiency measurement

The preparative method results in an heterogeneous system currently designated in the scientific literature as an 'emulsion'. As has been shown by many authors, it is not

* Registered Trademark, Rohm and Haas, U.S.A.

† PPO = 2,5-diphenyl oxazole; POPOP = *p*-bis (2,5-phenyl oxazolyl) benzene.

Table 2. Clinical studies with β -radiotracers in our laboratory.^a

		Tracer	Molecule
Compartmental studies	Total body water	Tritium	H ₂ O
	Extracellular space	Sulphur-35	SO ₄ ⁻
Metabolic studies	Calcium metabolism	Calcium-45	Ca ⁺
	Azathioprine	Sulphur-35	Imuran ^{®b}

^a I.N.S.E.R.M. U.90 (J.-L. Funck-Brentano) and Clinique Nephrologique (J. Hamburger) Hôpital Necker, Paris.

^b Imuran[®]: Burroughs Wellcome, U.S.A.

Table 3. Direct plasma sample preparation.

NaCl 9 g/l	2 ml	For plasma dilution
+		
Plasma	1 ml	
+		
S.E.M.	12 ml	Cooling (ice bath) Plasma + NaCl solution + S.E.M. Counting at 4°C
S.E.M.:	Scintillator emulsifying mixture Composition: toluene scintillator solution PPO 4 g/l POPOP 100 mg/l	
	This solution is mixed with Triton X-100 [®] in the proportion of 1 : 1 toluene : Triton X-100.	

possible with those systems to make use of the internal standard method for counting efficiency measurement. This method when applied to heterogeneous systems results in two values corresponding to the various phases constituting the system. Thus, we have used a method previously described by us as 'pseudo-internal' standardization.¹¹ This method is schematically represented in Table 4.

Two samples are prepared — the first one, as described above; the second, with the addition of a radioactive standard solution of known activity diluted in physiological saline. Thus, we obtained two samples with exactly the same physical structure and phase properties. The computation of detection efficiency is classical and is briefly presented in Table 4. The maximum counting efficiency is designated numerically as 1.00.

Quenching studies

For studying quenching, we have made use of two quenching factors:

1. B/C — ratio of the count rate in two channels B and C

Table 4.
Counting efficiency measurement by 'pseudo-internal' standardization.

Mixing order	I Plasma sample (P _I)	II P _I + radioactive standard of known activity
1	NaCl 9 g/l (2 ml)	Radioactive standard
2	Plasma P _I (1 ml)	1 ml plasma P _I solution
3	S.E.M. (12 ml)	12 ml S.E.M.

Counting efficiency computation $E(P_I)$ of the plasma sample (P_I).

N_I : Counting rate of the plasma sample P_I (c.p.m.)
 N_{II} : Counting rate of the standardized plasma (c.p.m.)
 D_{st} : Activity in d.p.m. of the radioactive standard solution
 $D(P_I)$: Activity in d.p.m. of the plasma sample P_I

$$E(P_I) = \frac{N_{II} - N_I}{D_{st}}$$

Activity in d.p.m. of the plasma sample P_I

$$D(P_I) = \frac{N_I}{E(P_I)}$$

2. E_1/E_2 – ratio of the count rate in two channels E_1 and E_2 of an external γ -emitter (caesium-137).

The discriminator levels are shown in Fig. 1. Using the SL 40 computer facilities, it is possible to determine simultaneously these two factors.

RESULTS

Detection efficiency

Counting efficiencies of the plasma samples labelled with tritium in the case of whole body water measurements, sulphur-35 for extracellular space and azathioprine metabolism and calcium-45 for metabolic studies are respectively:

1. For tritium, a great number of samples have detection efficiencies between 0.08 and 0.14. A few samples have efficiencies inferior or superior to these values. Finally, the extreme values observed were 0.02 and 0.18.
2. For ³⁵S-labelled plasma a great number of samples have detection efficiencies between 0.55 and 0.75. A few samples have values inferior or superior to these values. The extreme values observed for sulphur-35 detection efficiencies were 0.38 to 0.83.
3. For radioactive calcium-45 a great number of plasma samples have detection efficiencies between 0.63 and 0.83. A few samples have detection efficiencies inferior or superior to these values. Extreme values for calcium-45 detection efficiencies in plasma samples were 0.40 and 0.88.

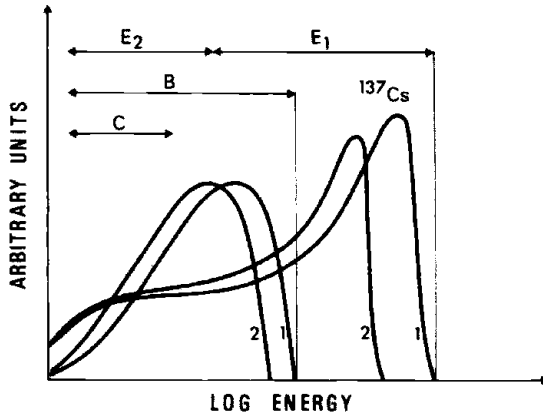


Fig. 1. Discriminator levels for quenching studies.

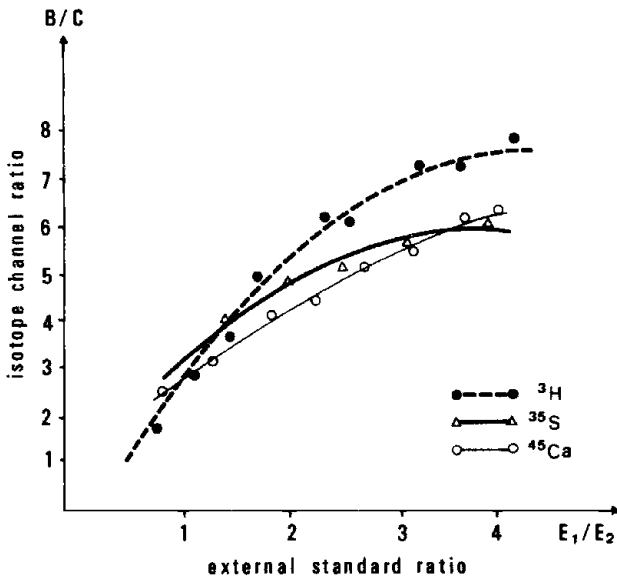


Fig. 2. Relationship between isotope channels ratio and external standard ratio.

Dynamic quench range of plasma samples

The results of counting efficiency measurement show that quenching is variable in plasma samples. Thus, we must choose a method for quench correction. The two classical methods are isotopes channels ratio and external standard channels ratio. A number of difficulties have been reported when using these two methods when applied to heterogeneous systems similar to the one used in this study.⁸⁻¹⁰ Bush¹⁰ recently pointed out that in those cases it is possible to check the validity of external standard ratio by studying the relationship between the factors B/C and E_1/E_2 . We have made use of this method and by

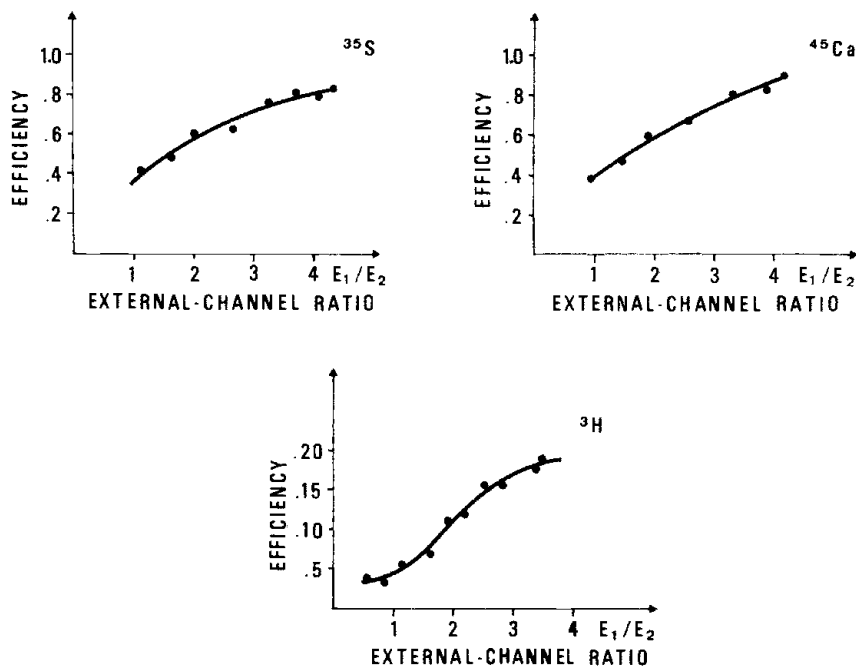


Fig. 3. Quenching dynamics of ^3H -, ^{35}S - and ^{45}Ca -labelled plasma samples as a function of the external standard channels ratio.

using the computer facilities of the SL 40 apparatus, we have obtained simultaneously these two factors for samples which have different counting efficiencies as measured by our 'pseudo-internal' method.

The results are shown in Fig. 2. There is a fixed relationship between the two quenching factors for the three isotopes used in our clinical studies. Thus, it is possible at least with our preparation system to make use of one or the other of these two quenching factors. Due to the easy measurement of the E_1/E_2 quenching factor and its better statistical accuracy we decided to use this factor to correct for quenching.

Curves of efficiency versus E_1/E_2 of ^3H -, ^{35}S - and ^{45}Ca -labelled plasma samples are represented in Fig. 3.

These quenching curves were obtained from clinical samples by measuring their counting efficiency and quenching factor. However, in view of the fact that the characteristics of our samples change after three days it is difficult to check long term instrument stability. Also we must add to our existing curve new values resulting from samples falling outside the curve limits. Computation parameters are modified according to these data. Thus, it was necessary to study the mode of quenching. In practice it is current to use a chemical quenching agent, for example carbon tetrachloride, water, acetone and so on. However, due to the various colours of the plasma samples which were green, yellowish or more or less red, it was expected that the quenching was of optical origin. At the last International Symposium on Liquid Scintillation Counting, Neary and Budd¹² stressed that the dynamics of quenching due to physical i.e. colour effects were different from those obtained with a chemical agent, at least with β -emitters having energies superior to tritium

Table 5. Preparation of samples for studying the origin of quenching.

Series	Added substances	Sample number								
		1	2	3	4	5	6	7	8	9
I Physical	Haemoglobin solution (μ l) (100 mg/ml)	0	2	5	10	20	50	100	200	500
	Hank's Liquid pH 7.4 and 6.8 (μ l)	0	50	100	200	350	500	1000	1500	2000
II Chemical	Pyridine (μ l)	0	20	50	100	300	500	1000	1500	—

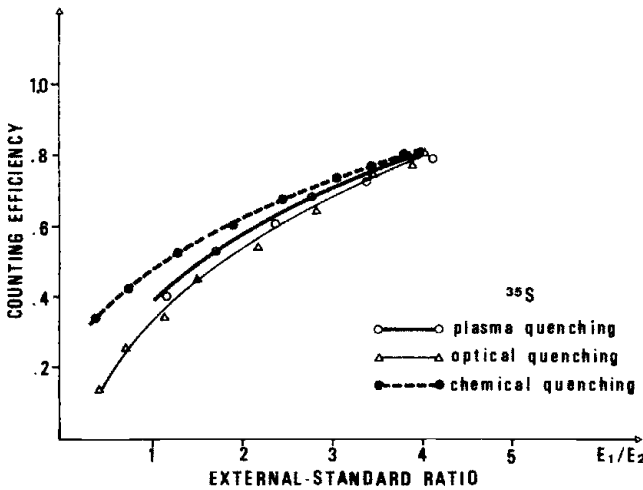


Fig. 4. Plasma quenching in comparison of optical and chemical quenching curves.

($E_{max} = 18$ keV). Thus, we have checked our hypothesis concerning the physical origin of plasma quenching. To do this, two series of samples were prepared as described on p. 224 but with physiological saline in place of plasma and containing two different quenching substances (see Table 5).

The first series was optically quenched by different volumes of haemoglobin solution at fixed concentration or Hank's Liquid at various pH levels. The second series was chemically quenched with different volumes of pyridine. We have chosen pyridine as the chemical quencher for its high water solubility. The results are shown in Fig. 4.

One may observe that the quenching in plasma samples is mostly of physical origin. For tritium the colour and chemical curves are identical as Neary and Budd have demonstrated,¹² but are not represented here. Thus, it is possible when counting labelled plasma samples to use a physical colour quench curve to obtain reliable results.

Table 6. Substances which may be found in human plasma and which interact with the liquid scintillation process by physical (i.e. colour) or chemical quenching.

Substances	Quenching effect	Wavelength maximum absorption ($m\mu$) ^a
Haemoglobin	Colour	275–600
Bilirubin	Colour	400–520
pH (Hydronium ion concentration)	Chemical	
Proteins	Chemical	
pO ₂ (Oxygen partial pressure)	Chemical	

^a Fluorescence spectra of PPO and POPOP: λ_{\max} = 380 and 420.

COMMENTS

In this study we have shown that when measuring labelled plasma samples by liquid scintillation counting, counting efficiencies vary, at least in the case of plasma samples for clinical studies. The origin of this variation is colour quenching induced by the different colours of the plasma samples. A number of molecules which have absorption spectra overlapping the emission spectra of the fluorescent scintillators may be found in human plasma. Other molecules which can interact with the process of energy transfer between solvent molecules are also present in human plasma. Table 6 shows a few examples of these molecules. These factors are present in all plasma samples, and most quenching substances which significantly influence counting efficiencies interact by optical absorption processes.

PRACTICAL CONSEQUENCES

The practical consequences of counting and the calculation of d.p.m. from c.p.m. in labelled plasma samples, in particular in the field of clinical studies, may be summarized as follows:

1. Detection efficiency does not remain constant. On the contrary, there may be a large range of efficiencies due to quenching.
2. This quenching is principally of physical origin (colour effects) and may be corrected by using a correction curve developed from a physical quenching agent.
3. It is possible to use the isotope channels ratio or the external standard ratio method to correct for quenching.

Finally, it is hoped that this study will contribute to the application of β -tracers in the field of clinical investigation and biological research.

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REFERENCES

- 1 A. E. Whyman, *Intern. J. Appl. Radiation Isotopes* **21**, 81 (1970).

- 2 A. Nadarajah, B. Leese and G. F. Joplin, *Intern. J. Appl. Radiation Isotopes* **20**, 733 (1969).
- 3 C. B. Oxby and P. A. Kirby, *Intern. J. Appl. Radiation Isotopes* **19**, 151 (1968).
- 4 R. H. Benson, *Anal. Chem.* **38**, 1353 (1966).
- 5 J. D. van der Laarse, *Intern. J. Appl. Radiation Isotopes* **18**, 485 (1967).
- 6 R. Lieberman, *Intern. J. Appl. Radiation Isotopes* **21**, 319 (1970).
- 7 P. H. Williams, *Intern. J. Appl. Radiation Isotopes* **19**, 377 (1968).
- 8 P. H. Springell, *Intern. J. Appl. Radiation Isotopes* **20**, 743 (1969).
- 9 H. E. Dobbs, *Intern. J. Appl. Radiation Isotopes* **19**, 155 (1968).
- 10 E. T. Bush, *Intern. J. Appl. Radiation Isotopes* **19**, 447 (1968).
- 11 C. Bader, J. Assailly, J. Chanard, J.-L. Funck-Brentano, *12e Table Ronde sur l'Exploration Fonctionnelle par les Isotopes Radioactifs*, Strasbourg, 1970.
- 12 M. P. Neary and A. L. Budd, in *The Current Status of Liquid Scintillation Counting* (Ed. E. D. Bransome), Grune and Stratton, New York, 1970.

DISCUSSION

H. Dobbs: At the risk of being labelled as a 'combustion obsessive' I would point out that plasma can be assayed very easily using combustion techniques. In our laboratories small volumes of plasma are evaporated to dryness in vacuum desiccators. The resultant dry powder is placed in rice paper cachets and burnt. This gives us very accurate and reproducible results. It also eliminates the possible inaccuracies due to surface adsorption which may be encountered when measuring tritiated compounds.